Vol. 5 No. 2 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Phytoconstituent Profiling and Biological Activity Assessment of *Hydrilla verticillata* Extract for Multifaceted Biotechnological Applications

Sumra Batool (Correspondence)
Department of Human Genetics & Molecular Biology, University of Health Sciences, Lahore, Pakistan. samrabatool.uhs@gmail.com

Zahid Hussain
Department of Zoology, University of Sindh, Jamshoro, Pakistan

Asma Hanif
Department of Botany, University of Karachi, Pakistan

Fozia Bibi
Department of Biology, Allama Iqbal Open University, Islamabad 44000, Pakistan.

Rasool Shah
Department of Zoology, Hazara University, Mansehra, Pakistan.

Author Details

Keywords: *Hydrilla verticillata*, phytochemicals, GC–MS, antioxidant activity, antimicrobial activity, bioactive compounds, aquatic medicinal plants

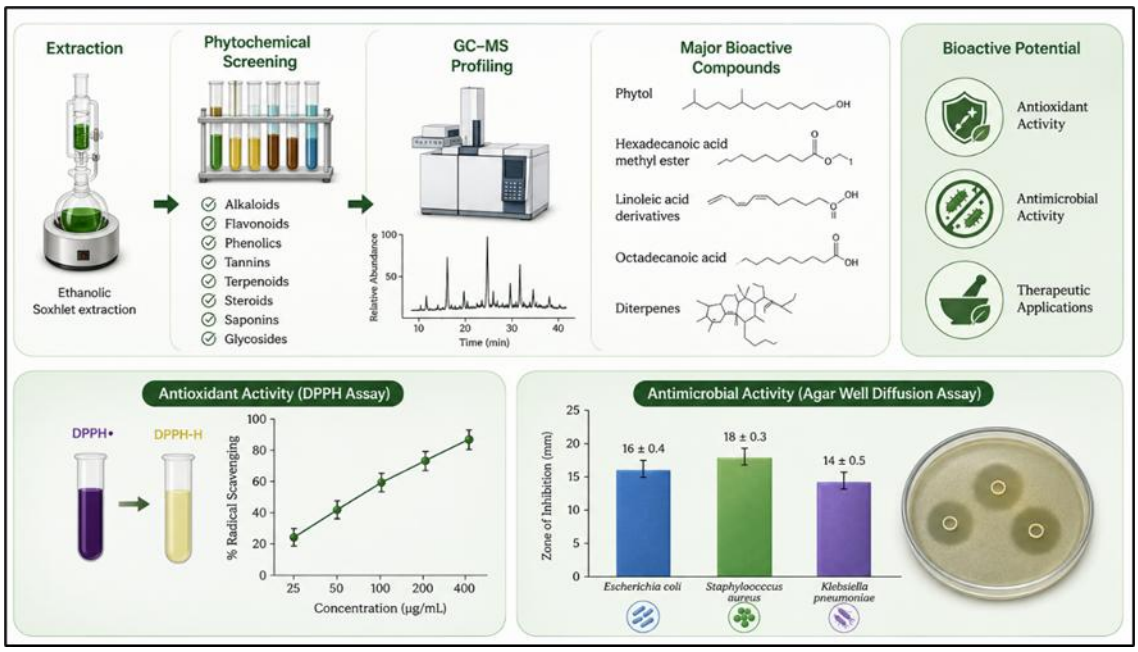
Received on 8 Apr 2026
Accepted on 10 May 2026
Published on 17 May 2026

Corresponding E-mail & Author*:

Sumra Batool
Department of Human Genetics & Molecular Biology, University of Health Sciences, Lahore, Pakistan. samrabatool.uhs@gmail.com

Abstract

Hydrilla verticillata is an aquatic macrophyte traditionally regarded as an invasive weed; however, recent investigations have demonstrated that it contains diverse phytochemicals with significant pharmacological potential. The present study aimed to evaluate the phytochemical composition of *H. verticillata* ethanolic extract and investigate its antioxidant, antimicrobial, and biological applications through qualitative phytochemical analysis, GC–MS profiling, and in vitro bioassays. Fresh plant material was collected, shade dried, powdered, and extracted using ethanol by Soxhlet extraction. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, terpenoids, steroids, saponins, and glycosides. GC–MS analysis identified major bioactive compounds including phytol, hexadecanoic acid methyl ester, linoleic acid derivatives, octadecanoic acid, and diterpenes. Antioxidant activity was assessed using DPPH radical scavenging assay, while antimicrobial efficacy was evaluated against selected Gram-positive and Gram-negative bacterial strains using agar well diffusion method. The ethanolic extract exhibited substantial antioxidant activity with concentration-dependent radical scavenging effects and showed appreciable antimicrobial inhibition against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The findings suggest that *H. verticillata* possesses considerable therapeutic potential and may serve as a promising source of natural bioactive compounds for pharmaceutical, nutraceutical, and biomedical applications.



Graphical Abstract

1. Introduction

Aquatic plants represent an underexplored reservoir of biologically active metabolites with remarkable medicinal importance. In recent years, increasing emphasis has been placed on the exploration of aquatic macrophytes as valuable sources of bioactive phytochemicals with therapeutic potential. These plants survive under unique environmental conditions characterized by fluctuating nutrient levels, water stress, microbial interactions, and oxidative challenges, which often stimulate the biosynthesis of diverse secondary metabolites (Maranho and Gomes, 2024). Such metabolites are known to exhibit significant biological activities including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antiviral, hepatoprotective, and anticancer effects. Consequently, aquatic plants are now being investigated as promising candidates for the development of novel pharmaceutical agents and functional biomaterials (Chellapandian et al., 2025).

Among these hydrophytes, Hydrilla verticillata has attracted increasing scientific attention due to its rich phytochemical composition and diverse pharmacological properties (Jain et al., 2025). *Hydrilla verticillata* (L.f.) Royle is a submerged perennial freshwater plant belonging to the family Hydrocharitaceae. The species is widely distributed across tropical and subtropical regions and is commonly found in ponds, lakes, canals, rivers, and irrigation channels (Patrick and Florentine, 2021). Although commonly considered a problematic aquatic weed in freshwater ecosystems, recent studies have demonstrated that the species contains several secondary metabolites with antioxidant, antimicrobial, anti-inflammatory, and anticancer potential (Unadkat and Parikh, 2021).

Medicinal plants synthesize numerous secondary metabolites such as alkaloids, flavonoids, phenolic acids, terpenoids, steroids, and glycosides, which play important roles in therapeutic interventions (Velu et al., 2018). These compounds are not directly involved in primary metabolic processes but serve crucial ecological and defensive functions in plants. Importantly, many of these metabolites possess potent therapeutic activities beneficial to human health (Zaynab et al., 2019). These bioactive molecules are known to protect biological systems against oxidative stress and microbial infections. Oxidative stress generated by reactive oxygen species (ROS) contributes to the development of chronic diseases, including diabetes mellitus, cardiovascular

disorders, cancer, and neurodegenerative conditions. Free radicals can induce cellular damage by oxidizing lipids, proteins, carbohydrates, and nucleic acids, ultimately leading to metabolic dysfunction and tissue injury (Phaniendra et al., 2015). Consequently, natural antioxidants from plants have become increasingly important in pharmaceutical and biomedical research (Bai et al., 2022).

In addition to oxidative stress, microbial infections remain a major global health challenge due to the increasing prevalence of multidrug-resistant pathogens. The emergence of antibiotic resistance among pathogenic bacteria has intensified the search for alternative antimicrobial agents from natural sources (Alara and Alara, 2024). Medicinal plants are considered promising candidates because their phytochemical constituents can inhibit microbial growth through multiple mechanisms, including disruption of cell membranes, inhibition of protein synthesis, and interference with microbial metabolic pathways. Therefore, the evaluation of antimicrobial activities of aquatic medicinal plants has become an important area of pharmacological research (Ashraf et al., 2023).

Previous reports on *H. verticillata* revealed the occurrence of phytol, fatty acid esters, phenolic compounds, and terpenoids possessing significant antimicrobial and antioxidant properties. However, comprehensive studies integrating phytochemical profiling and biological applications remain limited. Therefore, the present investigation was designed to perform phytochemical screening of *H. verticillata* extract, identify bioactive constituents using GC–MS analysis, evaluate antioxidant potential using DPPH assay, investigate antimicrobial efficacy against selected pathogenic bacteria, and assess the biological significance of identified compounds.

2. Materials and Methods

2.1 Collection of Plant Material

Fresh samples of Hydrilla verticillata were collected from freshwater ponds and irrigation channels. The collected material was thoroughly washed with distilled water to remove debris and contaminants. Plant samples were shade-dried for 10–15 days and pulverized into fine powder using a mechanical grinder.

2.2 Preparation of Plant Extract

Approximately 100 g of powdered plant material was subjected to Soxhlet extraction using 95% ethanol for 8 hours. The extract was filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator at reduced pressure. The crude extract was stored at 4°C until further analysis.

2.3 Preliminary Phytochemical Screening

Qualitative phytochemical analysis was conducted using standard biochemical procedures for detecting major secondary metabolites that include Mayer's test for alkaloids, Alkaline reagent test for flavonoids, Ferric chloride test for phenolics and tannins, foam test for saponins, Keller-Killiani test for glycosides, Salkowski test for steroids, and Liebermann–Burchard test for terpenoids.

2.4 GC–MS Analysis

Gas Chromatography–Mass Spectrometry analysis was performed using a PerkinElmer GC–MS system equipped with an Elite-5MS capillary column. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The injection temperature was 250°C. The oven temperature was adjusted to 60–280°C. The ionization energy was 70 eV with a scan range of 40–600 m/z. Compounds were identified by comparing mass spectra with NIST library databases.

2.5 Determination of Antioxidant Activity

Antioxidant activity was measured using the DPPH radical scavenging assay. Different concentrations of plant extract (20–100 µg/mL) were mixed with DPPH solution and incubated in darkness for 30 minutes. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer.

The percentage inhibition was calculated as:

$$\% \, Inhibition = \frac{A_0 - A_1}{A_0} \times 100$$

Where:

- A_0= control absorbance
- A_1= sample absorbance

2.6 Antimicrobial Activity

Antimicrobial activity was evaluated using the agar well diffusion method against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae.*

Sterile nutrient agar plates were inoculated with bacterial cultures. Wells were loaded with different concentrations of extract and incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters.

3. Results

3.1 Preliminary Phytochemical Screening

The ethanolic extract of *H. verticillata* demonstrated the presence of multiple biologically active phytochemicals.

Phytochemical Constituent	Presence
Alkaloids	+
Steroids	+
Terpenoids	+
Glycosides	+
Saponins	+
Tannins	+
Phenolics	+
Flavonoids	+

The abundance of flavonoids and phenolic compounds suggested strong antioxidant potential.

3.2 GC–MS Profiling of Bioactive Compounds

GC–MS analysis identified several important phytoconstituents with medicinal significance.

Compound Identified	Retention Time	Biological Activity
Phytol	18.42 min	Antioxidant, antimicrobial
Hexadecanoic acid methyl ester	16.35 min	Anti-inflammatory
Linoleic acid methyl ester	19.87 min	Hypocholesterolemic
Octadecanoic acid	20.12 min	Antimicrobial
Diterpene derivatives	22.31 min	Antioxidant
Ergosteroid compounds	24.14 min	Immunomodulatory

The predominant compound detected was phytol, which has been reported as a valuable diterpene alcohol with pharmaceutical significance.

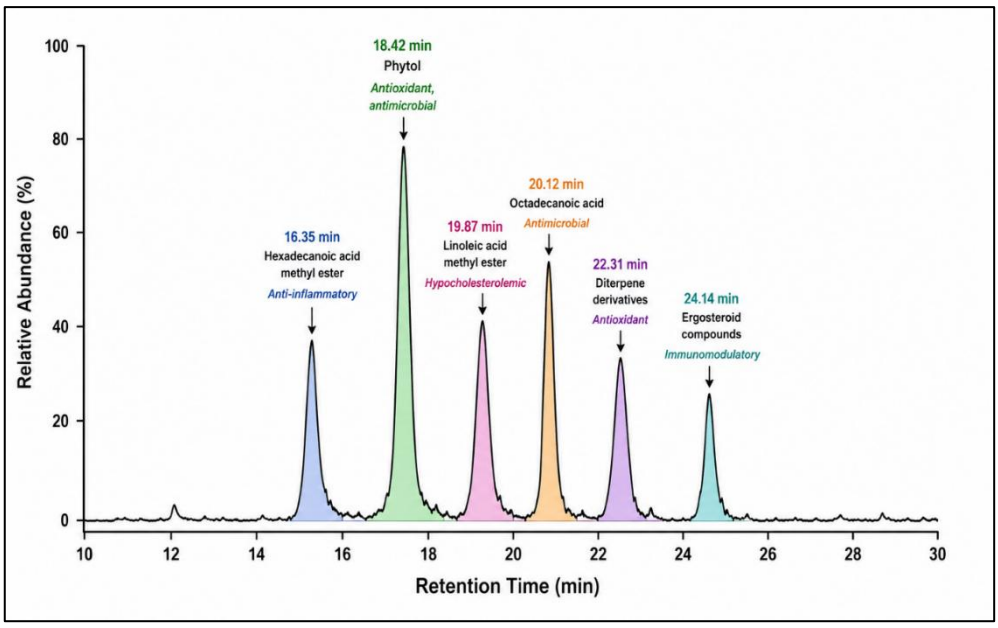


Figure 1. GC–MS analysis of *H. verticillata* bioactive compounds.

3.3 Antioxidant Activity

The extract demonstrated concentration-dependent DPPH radical scavenging activity.

Concentration (µg/mL)	% Inhibition
20	28.4
40	42.7
60	58.3
80	71.5
100	84.2

The highest antioxidant activity was observed at 100 µg/mL, indicating strong free radical scavenging capacity.

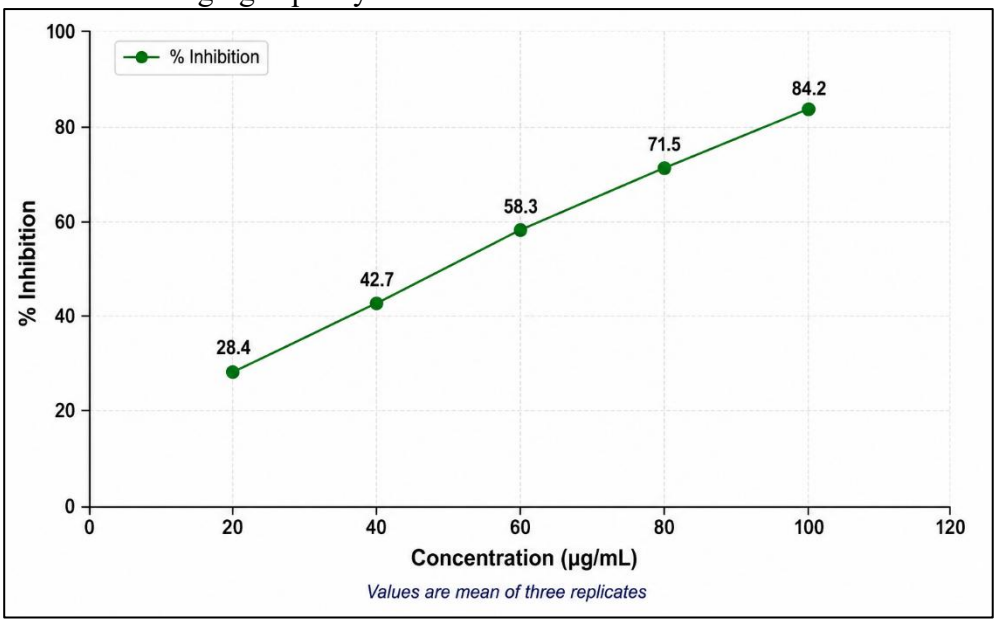


Figure 2. DPPH radical scavenging activity of *H. verticillata.*

3.4 Antimicrobial Activity

The ethanolic extract showed notable antibacterial activity.

Test Organisms	Zone of Inhibition (mm)

Escherichia coli	16 ± 0.4
Staphylococcus aureus	18 ± 0.3
Klebsiella pneumoniae	14 ± 0.5

The strongest inhibition was observed against *S. aureus*.

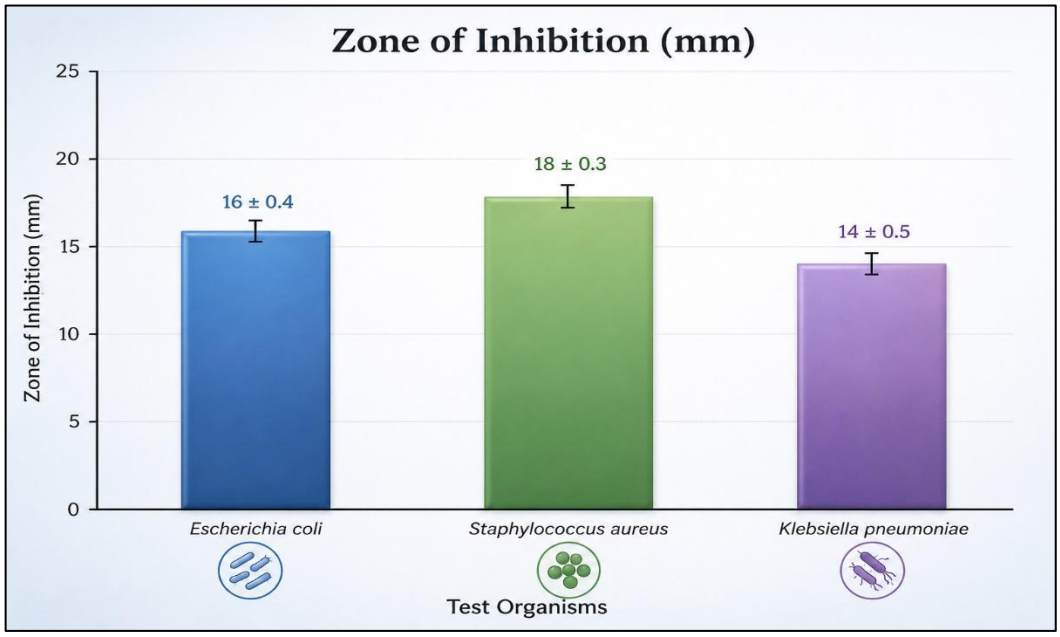


Figure 3. Antimicrobial activity of *H. verticillata* Ethanolic extract.

4. Discussion

The present investigation demonstrated that Hydrilla verticillata is a rich source of biologically active phytochemicals with significant therapeutic applications. Preliminary phytochemical screening confirmed the occurrence of alkaloids, flavonoids, phenolics, terpenoids, steroids, and glycosides, which are recognized for their medicinal relevance. A recent comprehensive review on hydrophytes highlighted that aquatic macrophytes possess unique phytochemical adaptations due to environmental stress conditions, enabling them to synthesize highly reactive antioxidant and antimicrobial metabolites with substantial pharmaceutical value (Alharthi et al., 2024; Behera et al., 2024).

The antioxidant activity recorded in the present work may be closely associated with the high concentration of phenolic constituents and flavonoids capable of scavenging reactive oxygen species (ROS). These compounds interrupt lipid peroxidation pathways and stabilize free radicals through electron donation mechanisms. Recent studies demonstrated that extracts of *H. verticillata* exhibit remarkable radical-scavenging efficiency against DPPH, ABTS, hydroxyl radicals, and superoxide radicals, suggesting their potential role in reducing oxidative stress–related disorders such as diabetes, cardiovascular diseases, neurodegeneration, and cancer. Furthermore, antioxidant peptides isolated from *H. verticillata* were recently reported to protect HepG2 cells against oxidative injury induced by hydrogen peroxide, thereby confirming the cytoprotective role of its natural bioactive constituents (Tang et al., 2024).

The GC–MS profile obtained in the current investigation revealed phytol as a major constituent of the ethanolic extract. Phytol has gained considerable scientific interest because of its multifunctional pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, antinociceptive, and anticancer activities. Recent reports demonstrated that phytol isolated from *H. verticillata* exhibited strong antibacterial activity and showed industrial relevance in biopolymer

production through polyhydroxyalkanoate synthesis (Petpheng et al., 2024). In addition, fatty acid derivatives such as hexadecanoic acid methyl ester and linoleic acid derivatives identified in the extract are known to possess membrane-disruptive antimicrobial effects and anti-inflammatory properties. These compounds may synergistically contribute to the observed biological activities of the plant extract.

The antibacterial activity observed against pathogenic microorganisms further confirms the medicinal importance of *H. verticillata*. Gram-positive bacteria appeared more susceptible to the extracts, likely because of the comparatively simple peptidoglycan-rich cell wall structure that allows easier penetration of phytochemicals, whereas Gram-negative bacteria possess an additional outer membrane acting as a permeability barrier. Similar findings have been reported in recent antimicrobial studies on aquatic medicinal plants, where phenolics, terpenoids, and fatty acid esters were considered major contributors to microbial growth inhibition (Petpheng et al., 2024). The antibacterial efficacy of the plant suggests its possible utilization as a natural alternative to synthetic antimicrobial agents, especially in the context of increasing antibiotic resistance worldwide.

Apart from pharmaceutical applications, the phytochemical richness of *H. verticillata* offers promising opportunities in nutraceuticals, cosmeceuticals, and functional food industries. Natural antioxidants and bioactive fatty acids derived from aquatic plants are increasingly being explored as ingredients in skin-protective formulations, anti-aging products, and dietary supplements. Additionally, the high biomass productivity and rapid growth rate of *H. verticillata* make it economically attractive for sustainable biotechnological exploitation (Evans and Wilkie, 2010).

Environmental applications of *H. verticillata* also deserve considerable attention. Recent studies have shown that the species can absorb heavy metals, excess nutrients, and organic pollutants from contaminated aquatic ecosystems, making it an efficient phytoremediation agent. Its interaction with microbial biofilms and aquatic nutrient cycles further highlights its ecological importance in wastewater treatment and aquatic ecosystem restoration (Duan et al., 2024). Thus, the plant possesses dual significance as both an ecological remediation resource and a medicinally valuable hydrophyte. Overall, the study establishes *H. verticillata* as a promising natural resource for bioactive compounds with broad-spectrum biological applications.

5. Conclusion

The present study successfully demonstrated that the ethanolic extract of Hydrilla verticillata contains diverse phytochemicals with significant biological activities. Preliminary phytochemical screening revealed the presence of therapeutically important metabolites including flavonoids, alkaloids, phenolics, terpenoids, steroids, and glycosides. GC–MS analysis identified phytol and fatty acid derivatives as major bioactive constituents. The extract exhibited remarkable antioxidant and antimicrobial activities, confirming its medicinal relevance. The findings suggest that *H. verticillata* could serve as a sustainable source of natural antioxidants and antimicrobial agents for pharmaceutical and biomedical applications. Further investigations involving purification of active compounds, molecular docking, toxicity evaluation, and in vivo pharmacological studies are recommended for future therapeutic development.

REFERENCES

Alara, J. A., & Alara, O. R. (2024). An overview of the global alarming increase of multiple drug resistant: a major challenge in clinical diagnosis. Infectious Disorders-Drug Targets Disorders, 24(3), 26-42.

Alharthi, F., Althagafi, H. A., Jafri, I., Oyouni, A. A. A., Althaqafi, M. M., Al-Hijab, L. Y. A., Al-Hazmi, N. E., Elagib, S. M., & Naguib, D. M. (2024). Phytochemical Composition and Bioactivities of Some Hydrophytes:

Antioxidant, Antiparasitic, Antibacterial, and Anticancer Properties and Mechanisms. Plants, 13(15), 2148.

Ashraf, M. V., Pant, S., Khan, M. H., Shah, A. A., Siddiqui, S., Jeridi, M., Alhamdi, H. W. S., & Ahmad, S. (2023). Phytochemicals as antimicrobials: prospecting Himalayan medicinal plants as source of alternate medicine to combat antimicrobial resistance. Pharmaceuticals, 16(6), 881.

Bai, L., Xu, D., Zhou, Y. M., Zhang, Y. B., Zhang, H., Chen, Y. B., & Cui, Y. L. (2022). Antioxidant activities of natural polysaccharides and their derivatives for biomedical and medicinal applications. Antioxidants, 11(12), 2491.

Behera, B., Meher, R. K., Mir, S. A., Nayak, B., & Satapathy, K. B. (2025). Phytochemical profiling, in vitro analysis for anti-inflammatory, immunomodulatory activities, structural elucidation and in silico evaluation of potential selective COX-2 and TNF-α inhibitor from Hydrilla verticillata (Lf) Royle. Journal of Biomolecular Structure and Dynamics, 43(2), 859-873.

Chellapandian, H., Jeyachandran, S., Park, K., & Kwak, I. S. (2025). Marine-derived functional biomaterials: advancements in biomedicine and drug delivery applications. Natural Product Communications, 20(6), 1934578X241302009.

Duan, R., Zhang, S., Jiang, S., Zhang, S., Song, Y., Luo, M., & Lu, J. (2024). Glufosinate-ammonium increased nitrogen and phosphorus content in water and shaped microbial community in epiphytic biofilm of *Hydrilla verticillata*. Journal of Hazardous Materials, 479, 135674.

Evans, J. M., & Wilkie, A. C. (2010). Life cycle assessment of nutrient remediation and bioenergy production potential from the harvest of hydrilla (*Hydrilla verticillata*). Journal of Environmental Management, 91(12), 2626-2631.

Jain, A. K., Barik, M., & Behera, B. (2025). Unveiling the Therapeutic Potential of Hydrocharitaceae Family: A Review of Phytochemistry and Pharmacology. International Research Journal of Multidisciplinary Scope, 6(01), 927-937.

Maranho, L. T., & Gomes, M. P. (2024). Morphophysiological adaptations of aquatic macrophytes in wetland-based sewage treatment systems: Strategies for resilience and efficiency under environmental stress. Plants, 13(20), 2870.

Patrick, A. E., & Florentine, S. (2021). Factors affecting the global distribution of Hydrilla verticillata (L. fil.) Royle: A review. Weed Research, 61(4), 253-271.

Petpheng, B., Mudtaleb, B., Piboon, W., Paichid, N., & Sangkharak, K. (2024). The extraction of phytol from *Hydrilla verticillata* using ultrasonic-assisted extraction (UAE), the analysis of antibacterial activity, and the utilization of residual extract for polyhydroxyalkanoate (PHA) production. Biomass Conversion and Biorefinery, 14(18), 22007-22017.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. Indian journal of clinical biochemistry, 30(1), 11-26.

Tang, Y., Liang, F., Yan, Y., Zeng, Y., Li, Y., & Zhou, R. (2024). Purification and identification of peptides from Hydrilla verticillata (Linn. f.) Royle with cytoprotective and antioxidative effect against H2O2-treated HepG2 Cells. Journal of Agricultural and Food Chemistry, 72(8), 4170-4183.

Unadkat, K., & Parikh, P. (2021). Therapeutic potential of some aquatic macrophytes: An overview. Trends in Medical Research, 16(1), 1-6.

Velu, G., Palanichamy, V., & Rajan, A. P. (2018). Phytochemical and pharmacological importance of plant secondary metabolites in modern medicine. In Bioorganic phase in natural food: an overview (pp. 135-156). Cham: Springer International Publishing.

Zaynab, M., Fatima, M., Sharif, Y., Zafar, M. H., Ali, H., & Khan, K. A. (2019). Role of primary metabolites in plant defense against pathogens. Microbial Pathogenesis, 137, 103728.